iGEM 2016: Pittsburgh
Week 3 Lab Notebook

Monday, June 6

Lab meeting
Transformation, iGEM protocol
amilCP BBa_K592009 Kit Plate 1, 19E
T7 RBS (strong) BBa_K525998 Kit Plate 1, 3M
Terminator BBa_B0015 Plate 3, 3F

66 uL competent cells per tube (one 68 uL)
5 uL resuspended DNA per tube
To resuspend: pierce foil w/ tip, then add dH2O
(Ice for > 30 min, b/c water bath not at temp yet)
Incubate 2 PM - 4 PM (~20 C)
Plate 100 uL each (LB + CM)

OUTREACH
Praneeth emailed Steve (tissue engineering camp)

Tuesday, June 7

Many more colonies from transformation
5 mL liquid cultures in 15 mL centrifuge
5 mL LB + 5 uL CM (1:1000)
Each person makes one (4*3 = 12)
Into room temp shaker 1:40 PM

Wednesday, June 8

Mini-prep of liquid cultures
Wash solution + 170 mL, 100% ethanol
Transfer cells into 1.5 tube to pellet @ 8000 rpm
1 liquid culture * 3 plasmids = 3 (for glycerol stocks)
From 6/6 plates
Into shaker at 12:15 PM
40% glycerol solution
Final volume 25 mL: 20 mL 50% glycerol and 5 mL H2O (10 mL glycerol)

Thursday, June 9

Long-term bacterial stocks from yesterday's liquid cultures -- in -80 box with Top 10 cells
DNA concentrations from yesterday's mini-prep
Blank w/ elution buffer

(unit: ng/uL)	T7-RBS	amilCP	terminator
CC	74.6	123.1	88.6
MLK	108.5	109.0	88.5
PP	76.3	245.2	96.9
AN	64.3	118.2	58.7

Restriction digest

T7-RBS MLK
2 uL SpeI
2 uL 10X buffer
1 ug * 1000 ng/ug * uL/108.5 ng = 9.22 uL DNA
6.78 uL H2O
20 uL Total

T7-RBS PP
2 uL SpeI
2 uL 10X buffer
13.1 uL DNA
2.9 uL H2O
20 uL Total

amilCP CC
1.5 uL SpeI
1.5 uL XbaI
3 uL 10X buffer
2 ug = 16.25 uL DNA
7.75 H2O
30 uL Total

amilCP PP
1.5 uL SpeI
1.5 uL XbaI
3 uL 10X buffer
8.16 uL DNA
15.84 uL H2O
30 uL Total

Terminator CC
2 uL XbaI
2 uL 10X buffer
11.29 uL DNA
4.71 uL H2O
20 uL Total

Terminator PP
2 uL XbaI
2 uL 10X Buffer
10.32 uL DNA
5.68. uL H2O
20 uL Total

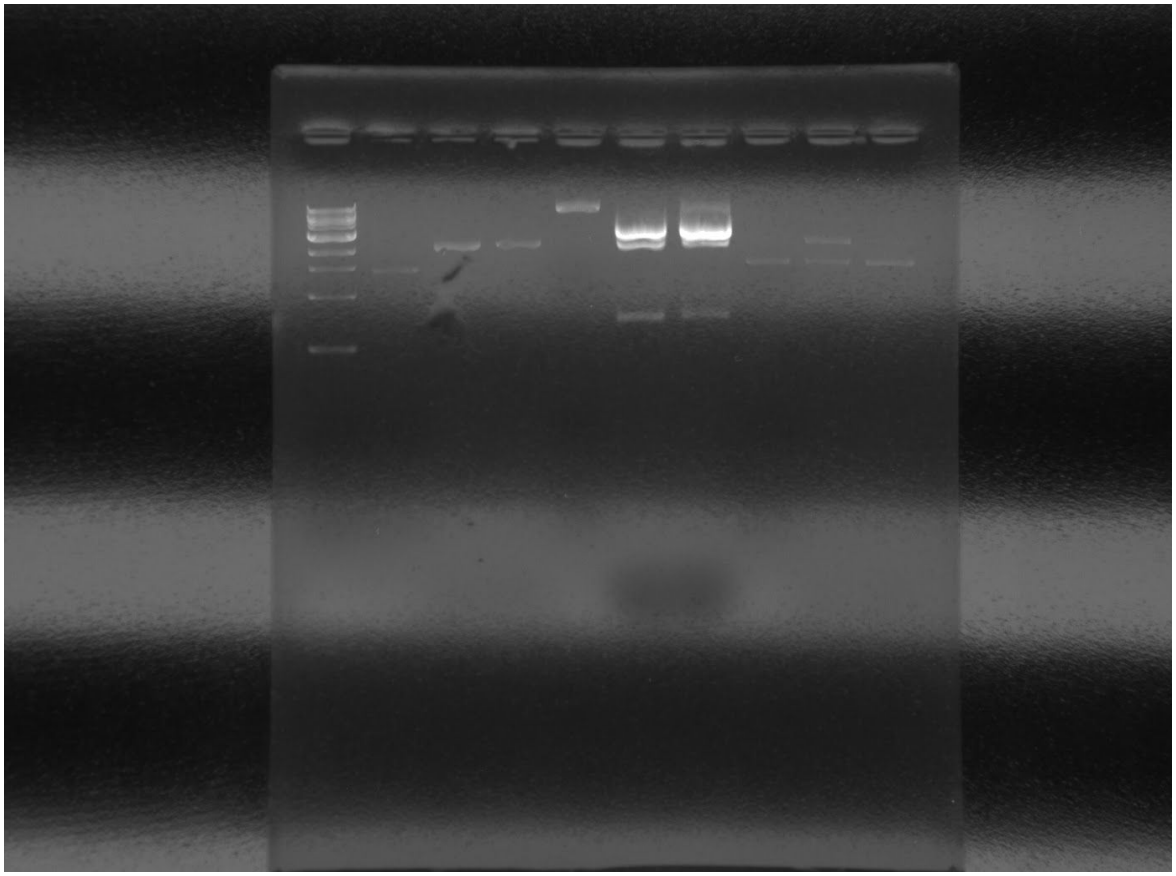
General Case (20 uL) 2 uL enzyme
10X buffer → 1X
Plasmid 1-2 ug
Nuclease-free H2O to volume

Incubate @ 37 C
Incubate @ 80 C for 20 min (deactivate enzymes)

Gel electrophoresis

1% agar in 1X TAE buffer (50 mL, .5 g agarose, microwave)
Add 5 uL ethydium bromide
Pour gel

Lane 1 - 3-4 uL ladder, 4 uL loading dye
Lane 2 - 1 uL T7 MLK control (uncut), 2 uL dye
Lane 3 - 1 uL T7 MLK in buffer
Lane 4 - 1 uL T7 PP in buffer
Lane 5 - 1 uL amilCP control (uncut), 2 uL dye
Lane 6 - all amilCP CC in buffer
Lane 7 - all amilCP PP in buffer
Lane 8 - 1 uL terminator control (uncut), 2 uL dye
Lane 9 - 1 uL terminator CC in buffer
Lane 10 - 1 uL terminator PP in buffer
T7-RBS good, amilCP okay, terminator questionable--XbaI issue?



Purify linearized plasmids (T7 and terminator) (Maya)

Concentrations: Terminator CC -- 43.9 ng/uL
Terminator PP -- 39.0 ng/uL
T7-RBS MLK -- 58.2 ng/uL
T7-RBS PP -- 45.8 ng/uL

Cut and purify amilCP bands (Praneeth)

Concentrations: 6.6 ng/uL
9.0 ng/uL

Sequencing reaction (Pitt's Genomics Research Core) (Claire)

Sample submission protocol

15 uL

8 pmoles of primer (10 picomolar) = 100 uL H2O + .1 uL 10 mM

1 ug DNA -- 10 mM

T7 MLK *Forward* -- CC1
9.22 uL DNA
1 uL 10 pM forward primer
4.78 uL H2O

Backward -- CC2
9.22 uL DNA
1 uL 10 pM backward primer
4.78 uL H2O

amilCP PP *Forward* -- CC3
4.08 uL DNA
1 uL 10 pM forward primer
9.92 uL H2O

Backward -- CC4
4.08 uL DNA
1 uL 10 pM backward primer
9.92 uL H2O

Terminator PP *Forward* -- CC5
10.32 uL DNA
1 uL 10 pM forward primer
3.68 uL H2O

Backward -- CC6
10.32 uL DNA
1 uL 10 pM backward primer
3.68 uL H2O

Friday, June 10

Reattempt Restriction Digest of Terminator with XbaI-- 30 uL

3 uL XbaI
3 uL 10X buffer
11.30 uL MLK terminator (1 ug)

12.7 uL nuclease-free water

Ligation reaction

Dephosphorylate T7-RBS

DNA = 10 uL

Add 0.5 uL rSAP (recombinant Shrimp Alkaline Phosphatase)

37 C for 30 minutes

65 C for 5 min

Ligation

Control (no insert)	Plasmid: 1 uL with 58.2 ng/uL Insert : 0 T4 buffer: 2 uL T4 DNA Ligase: 1 uL H2O up to 20: 16 uL
3:1	Plasmid: 1 uL with 58.2 ng/uL Insert @ 50 ng: 5.56 uL of 90 ng/uL Buffer: 2 uL T4 DNA Ligase: 1 uL H2O: 10.44 uL
~5:1	Plasmid: 1 uL of 58.2 ng/uL Insert @ 81 ng: 1.7 mL of 9 ng/uL and 9.1 mL of 6.6 ng/uL Buffer: 2 uL Ligase: 1 uL H2O: 2.46 uL

T4 Ligase should be added last

Room temperature for 10 min, heat inactivate at 65 C for 10 min

Transformation

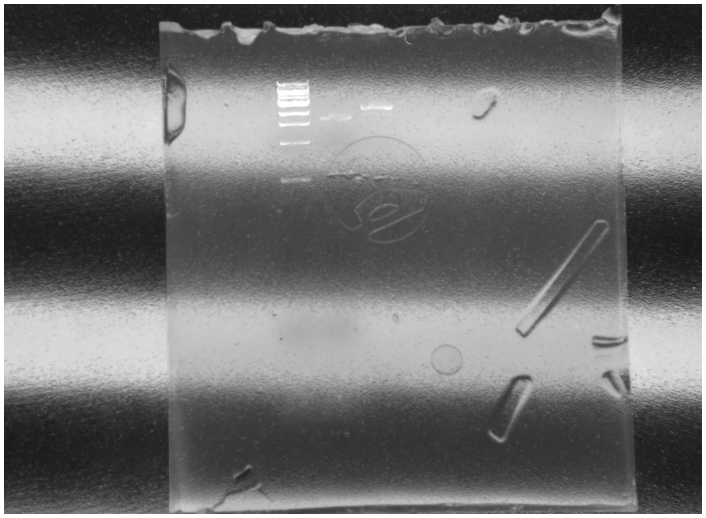
Use 5 uL of each with Cheryl's Top 10 cells (200 uL aliquot, so 66.66 uL for each)

66 uL of cells in each tube, 5 uL of control, 3:1, and 5:1 solutions

Gel electrophoresis of terminator (MLK)

1 kb ladder (lane 3), uncut (lane 4) and cut (lane 5)

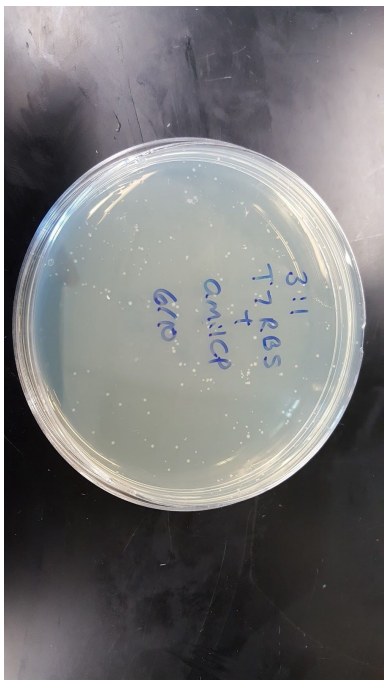
Gel run for 45 minutes (seems to work)

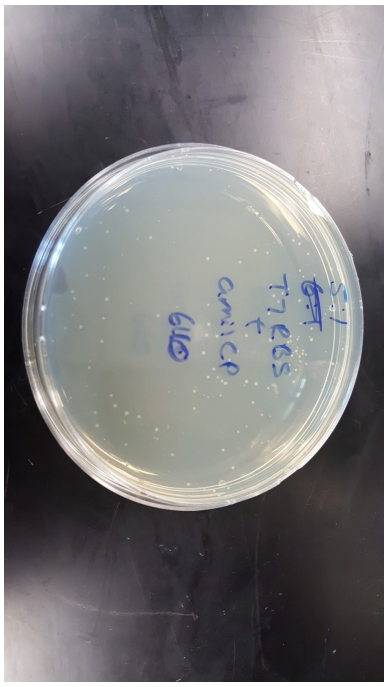


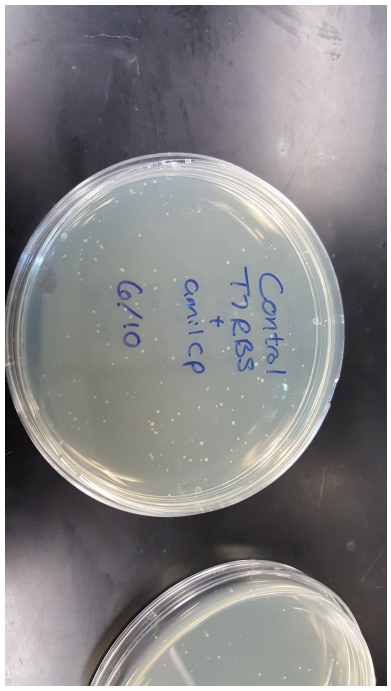
Phosphatase (rSAP) added to MLK terminator

Saturday, June 11

Remove colonies from incubator (Maya)







Sunday, June 12

Liquid cultures of T7 -- amilCP colonies (10 total cultures, 5 each of 3:1 and 5:1) (Praneeth)